DESCRIPTION

METHOD OF EVALUATING DRUG SENSITIVITY BY ANALYZING THE MU-OPIOID RECEPTOR GENE

Technical Field

The present invention relates to a method of evaluating drug sensitivity by analyzing a mu-opioid receptor gene.

Background Art

Stimulants such as methamphetamine exert its action by activating monoamine neurotransmission, and it is considered that in particular dopamine is most directly involved in an action of reinforcing a stimulant (Document 1). It is generally well known that the dopamine nervous system and the opioid nervous system interact with each other. Heretofore, it has been reported that an opioid receptor agonist regulates dopamine metabolism in the nerve terminal to induce dopamine release to a synaptic cleft (Document 2), and it attenuates methamphetamine-induced alterations in dopamine neurotransmission (Documents 3 and 4). Further, in the recent studies using knockout animals and the like, it is shown that there is a close interaction between

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both nervous systems (Documents 5 to 7). Therefore, it is considered that alterations in the opioid receptor function may cause individual differences in susceptibility to stimulant addiction and stimulant-induced psychotic-like symptoms.

On the other hand, it has been revealed that a mu-opioid receptor plays a very important role for an endogenous opioid peptide or a narcotic analgesic agent to develop an analgesic action and various side effects such as development of addiction, development of resistance and withdrawal symptoms. Further, recently, it became known that a mu-opioid receptor is also involved in addiction to a drug other than narcotic analgesic agents or alcohol or a part of mental diseases (Documents 8 to 11).

In particular, it is believed that a mu-opioid receptor gene polymorphism may have an influence on induction or suppression of development of addiction caused by an opioid drug or a nonopioid drug. Heretofore, in studies on mu-opioid receptor gene polymorphisms, the existence of polymorphisms mainly in translation regions, 5'UTR and a part of introns is shown using genes mainly of Europeans and Americans (Caucasian African-American) (Documents 12 to 14). In particular, as for a single nucleotide polymorphism A118G that causes an amino acid

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mutation, there is a report that the binding affinity of β-endorphin, which is one of endogenous ligands, increases in a mutant form of receptor which has been expressed in a cultured cell system, which has attracted attention. Further, mainly A118G has been investigated for narcotic or alcohol addiction in various races, however, the presence or absence of correlation varies depending on researchers, and the present situation is that a unified conclusion has not been achieved yet (Documents 15 to 21). On the other hand, a number of studies on the relationship between genetic factors of diseases or pathologic conditions and human gene polymorphisms have been carried out. However, many of them were analyses carried out using genomes of Europeans and Americans. It is known that the presence or absence of gene polymorphism and occurrence frequency greatly vary depending on races, therefore, it is essential that an analysis be carried out using Japanese genomes upon studying Japanese diseases or pathologic conditions and genetic factors.

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Disclosure of the invention

An object of the present invention is to provide a method of predicting individual differences in susceptibility to development of methamphetamine addiction from which many patients suffer among stimulant addictions and stimulant-induced psychotic-like symptoms using a mu-opioid receptor gene polymorphism.

The inventor made intensive studies and as a result, found that several novel mu-opioid receptor gene polymorphisms and elucidated that the gene polymorphisms are different between methamphetamine-addicted patients and normal subjects, thus the present invention has been completed.

That is, the present invention is as follows.

(1) A method of evaluating drug sensitivity, which comprises linking a gene polymorphism in a mu-opioid

receptor gene or a haplotype comprising the gene polymorphism to individual drug sensitivity.

In the present invention, as the above-mentioned gene polymorphisms, at least one polymorphism selected from the group consisting of single nucleotide polymorphisms (SNP), insertion polymorphisms, deletion polymorphisms and nucleotide repeat polymorphisms. Further, as the above-mentioned gene polymorphisms, polymorphisms shown in Table 4 can be exemplified. Further, the above-mentioned haplotype is, for example, a haplotype shown in Table 5 or 8.

As the above-mentioned drug, a mu-opioid receptor function modulator, for example, at least one member selected from the group consisting of methamphetamine methylenedioxymethamphetamine, amphetamine, dextroamphetamine, dopamine, morphine, DAMGO, codeine, methadone, carfentanil, fentanyl, heroin, cocaine, naloxone, naltrexone, nalorphine, levallorphan, pentazocine, buprenorphine, oxycodone, hydrocodone, levorphanol, etorphine, dihydroetorphine, hydromorphone, oxymorphone, ethanol, methanol, diethyl ether and tramadol can be exemplified.

(2) A method of determining a type and/or an amount of a drug to be administered to an individual by using as an index, a result evaluated by the above-mentioned

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method.

(3) A method of predicting a side effect of a drug to be administered to an individual by using as an index, a result evaluated by the above-mentioned method.

(4) An oligonucleotide comprising a sequence of at least 10 bases containing the 51st base in a nucleotide sequence represented by any of SEQ ID NOS: 1 to 98 or a sequence complementary to this.

Preferably, the above-mentioned oligonucleotide has a length of 10 to 45 bases containing the 51st base.

(5) An oligonucleotide selected from the group consisting of the nucleotide sequences represented by SEQ ID NOS: 1 to 98 and nucleotide sequences complementary to these.

(6) A microarray, in which the above-mentioned oligonucleotide has been immobilized on a support.

(7) A kit for evaluating drug sensitivity including the above-mentioned oligonucleotide and/or the above-mentioned microarray.

According to the present invention, a novel mu-opioid receptor gene polymorphism is provided. By using the polymorphism of the present invention, individual differences in sensitivity to a drug can be evaluated. By this method of evaluating sensitivity, an

appropriate prescribed amount of a narcotic drug such as morphine can be found.

Brief Description of the Drawings

Fig. 1 is a schematic view of a mu-opioid receptor gene (an analyzed range and results of linkage disequilibrium analysis among major polymorphisms).

Best Mode for Carrying Out the Invention

Hereinafter, the present invention will be described in detail.

1. Outline of the present invention

The present inventor identified many novel gene polymorphisms by analyzing mu-opioid receptor gene polymorphisms for normal subjects. The human mu-opioid receptor mRNAs are encoded by exons 1 to 4. The 5' untranslated region is present upstream of exon 1. Introns 1 to 3 are present between exons 1 and 2, 2 and 3, and 3 and 4, respectively, and the 3' untranslated region is present downstream of exon 4. As for the gene polymorphisms of the present invention, a number of novel polymorphisms different from the published polymorphisms are contained in these introns, exons and the like. Many of the SNPs found in intron 3 and the 3' untranslated region among the novel gene polymorphisms

of the present invention are in complete linkage disequilibrium. Here, the linkage equilibrium means a case where the relationship between two polymorphisms on the chromosome is independent, and the linkage disequilibrium means a case where a polymorphism is linked to the other polymorphism thereby deviating from the equilibrium situation according to Mendel's law of independence.

Further, the present inventor systematized the information of a group of SNPs according to haplotypes by using the above-mentioned SNP information. The haplotype means a genetic structure of such as genes or SNPs located in the vicinity of each other in one allele of a set of alleles (a gene derived from one of the parents). SNPs located in the vicinity on a genome are inherited in a block of strong linkage disequilibrium (a haplotype block). In other words, a haplotype also refers to a combination of the arrangement of the same gene in this haplotype block. In the case where several SNPs appear in association with a certain phenotype (for example, occurrence of a disease), even if not all the SNPs are typed, by analyzing several SNPs constituting a haplotype, a relationship between the genotype and the phenotype of a patient can be elucidated. The present inventor could estimate four haplotype blocks from the

results of the haplotype analysis.

Disease occurrence or drug sensitivity is not associated with only one polymorphism, but is associated on a haplotype unit in many cases. Further, it is very difficult to elucidate an unknown polymorphism present outside the untranslated region. Therefore, a haplotype is used for finding a relationship between a polymorphism and a disease or drug sensitivity. Accordingly, the present invention also provides a method using not only the above-mentioned SNP but also a haplotype as a gene polymorphism to be used for evaluating drug sensitivity.

The present inventor compared the SNP frequencies typified by the four estimated haplotype blocks elucidated from the results of the haplotype analysis between normal subjects and methamphetamine-addicted patients, and found that there was a difference in the occurrence frequencies between both groups, and in particular, the difference between both groups was evident when comparison was carried out after performing classification according to stimulant-induced psychotic-like symptoms.

Accordingly, by analyzing SNPs or haplotypes elucidated in the present invention, it becomes possible to evaluate individual drug sensitivity. This drug sensitivity will be important information upon

determining the type or amount of a drug. In particular, a stimulant or morphine may cause a big social problem depending on the usage, therefore, it is extremely useful to know in advance an appropriate amount of a drug to be administered to an individual before administering the drug.

2. Mu-opioid receptor gene polymorphism

The human mu-opioid receptor gene polymorphism of the present invention include mainly a single nucleotide polymorphism (SNP), an insertion/deletion polymorphism, and a polymorphism caused by the difference in the number of repeats of base sequence.

The SNP (SNPs) means a polymorphism caused by replacing a specific one base of a gene with another base. The insertion/deletion polymorphism means a polymorphism caused by deletion/insertion of one or more bases. Further, the polymorphism caused by the difference in the number of repeats of base sequence (nucleotide repeat polymorphism) include a microsatellite polymorphism (the number of bases: about 2 to 4 bases) and a VNTR (variable number of tandem repeat) polymorphism (repeated bases: several to several tens of bases) according to the difference in the number of repeated bases, and means a polymorphism in which the

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number of repeats varies depending on individuals.

The information of human mu-opioid receptor gene polymorphisms elucidated by the present invention is shown in the following Table 1, Table 2 and Table 3. In these tables, single nucleotide polymorphisms, insertion/ deletion polymorphisms, and polymorphisms caused by the difference in the number of repeats of base sequence are included.

The present invention provides an oligonucleotide containing a gene polymorphism shown in Table 1, Table 2 and Table 3 among mu-opioid receptor genes. In the present invention, a method of obtaining gene polymorphism information shown in Table 1, Table 2 and Table 3 is as follows.

Genomic DNA is purified from a blood specimen collected from a human using the phenol method and the like. At this time, a commercially available genomic DNA extraction kit such as GFX Genomic Blood DNA Purification Kit or a device may be used. Then, the genomic DNA is divided into several regions and amplified by the PCR method using the resulting genomic DNA as a template, thereby to prepare a template DNA for sequencing. The present invention is directed to a gene polymorphism, therefore, it is preferred that an enzyme with as higher fidelity as possible is used in the PCR method. As for

the 5' untranslated region (5' UTR and 5' flanking region) of the mu-opioid receptor, a region up to 6000 bp, preferably 5800 bp upstream from the start codon is divided into 2 to 4 regions, and PCR amplification is carried out. As for exon 1, exon 2-intron 2-exon 3, exon 4, and a part of intron 1 or 3, the full length is amplified by the PCR method for each region. The exon 4 may contain a region of about 3000 bp downstream from the stop codon. As for the 3' untranslated region, a region up to 14000 bp, preferably 13900 bp downstream from the stop codon is divided into 4 to 6 regions, and amplification is carried out by PCR. The nucleotide sequences of the full regions of these PCR fragments are analyzed such that a segment of about 500 bp was decoded by the sequencing method at a time using primers designed based on the sequence information published in GenBank, whereby a gene polymorphism of interest can be obtained. The thus obtained polymorphism information is shown in Table 1.

Table 1: SNP found on genome of Japanese normal subjects and polymorphism frequencies thereof

Position	SNP name	Amino acid substitution	Allelic frequency	Sample size	Reported Allelic frequency
5'flanking region and 5'UTR	A-5580G		<1.5%	44	
	C-5342T		<1.5%	44	
	A-5308G		<1.5%	44	
	C-5236T		<1.5%	44	
	G-5026A		<1.5%	44	
	G-4936A		<1.5%	44	
	C-4504A		<1.5%	44	
	T-2694G		<1.5%	44	
	A-2693C		<1.5%	44	
	T-2683C		<1.5%	44	
	T-2402C		<1.5%	44	
	C-1609T		<1.5%	44	
	G-696T		<1.5%	44	
	G-490T		<1.5%	44	
Exon 1	**A118G**	Asn40Asp	45.3%	213	7.5 - 25.8 %
Intron 1	IVS1-A4980G	-	2.7%	187	
	IVS1-A4910G	-	2.7%	187	
	IVS1-G4690A	-	<1.5%	113	
	IVS1-T4591G	-	2.7%	113	
	IVS1-T4456G	-	<1.5%	113	
Intron 2	IVS2+G31A	-	2.6%	232	4.2%
	IVS2+G518A	-	<1.5%	232	
	IVS2+G691C	-	81.9%	232	42.5 - 53.3%
Intron 3	IVS3+G5807A	-	44.4%	179	
	IVS3+G5953A	-	13.1%	179	
	IVS3+A6151G	-	92.5%	179	
	IVS3+A8449G	-	9.2%	179	
	IVS3+C8497T	-	31.3%	179	
	IVS3-G8804A	-	9.5%	179	
3'UTR	TAA+G886A	-	10.8%	97	
	TAA+T1360C	-	<1.5%	97	
	TAA+T1371C	-	10.8%	97	
	TAA+G1670A	-	11.4%	44	
	TAA+G1709A	-	5.7%	44	
	TAA+C2007T	-	11.4%	44	
	TAA+A2109G	-	8.4%	179	
	TAA+A2274G	-	11.7%	179	
	TAA+G2287A	-	90.8%	179	
	TAA+G2395C	-	8.4%	179	

In Table 1, "Position" means a position on the genome of a mu-opioid gene, and indicates 5' or 3' untranslated region, exon or intron. "SNP name" is the name of SNP at a position on the genome, and given by the present inventor. Basically, an alphabet of A, G, C or T is given before and after tree- or four-digit number and symbol, respectively, thus which base is involved in SNP can be identified. For example, "A-5580G" indicates a polymorphism in which the base 5580 bp upstream (5' side) of the start codon is changed between A and G. "IVS1-A4980G" in the column "Intron-1" indicates a polymorphism in intron 1, in which, assuming that the base which is in intron 1 and in contact with exon 2 is 1, the base 4980 bp upstream thereof has been changed from A to G. In the same manner, "TAA+G886A" in the column "3' UTR" indicates a polymorphism in which the base located at the 3' side of 886 bp from a stop codon, TAA, has been changed from G to A.

Table 2: Polymorphism found in 3' untranslated region
of mu-opioid receptor gene and linkage disequilibrium

TAA+	reported	major allele	minor allele	LD	TAA+	reported	major allele	minor allele	LD
886	A	G	A	*	8386	A(13)	A(16)	A(13)	*
1360	T	T	C		9000	C	C	T	
1371	T	T	C	*	9564	A	G		
1670	A	G	A	*	9669	G	A	G	*
1709	G	G	A		9716	T	T	A	
2007	C	C	T	*	9839	T	G	T	*
2026	G	G	A		9994	C	A	C	*
2109	A	A	G	*	10083	C	C	A	
2274	A	A	G		10223	A(4)	A(3)	A(4)	*
2287	G	A	G	*	10247	A	T	A	*
2395	G	G	C	(*)	10535	A	G		
2458	G	G	C		10704	G	G	A	
2482	T	T	C		10752	T	G	T	*
2497	G	G	A		11100	C	T		
2656	G	G	T	*	11129	C	A	C	*
2714	C	A			11132	CA(17)	CA(17)	CA(14)	*
2820	G	G	T	*	11133	A	A	G	
2907	G	G	T		11368	TCTC	-	TCTC	*
3423	T	C			11411	T	T	C	
4026	A	G			11431	T	C	T	*
4585	A(6)	A(5)	A(6)		11449	-	TTTC	-	*
4861	A	A	C		11541	G	G	A	*
5359	A	A	G		11602	A	C	A	*
6074	A	A	C		11650	C	T		
6866	T	T	G	*	11918	C	C	T	*
6922	C	G	C	*	11956	A	C		
7075-7396	322 bp	-			12143	A	A	G	*
7427	C	C	T	*	12630	A	G		
7483	A	-			12681	T	T	C	*
7536	T	C	T	*	12831	T	C		
7589	A	G	G		12834	G	C		
8116	C	T	C	*	13236	T(15)	T(14)	T(15)	
8165	C	T	T		13971	G	T		
8281	G	A	A						

The distance indicates the number of bases from a
stop codon (TAA). "reported" indicates a genotype in a

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genebank sequence, "major" and "minor" indicate a major genotype and minor genotype on the Japanese genome, respectively. The numerical value in a parenthesis in the table means the number of repeats of the base before the numerical value, and "-" means deletion or insertion. * in the column of LD (Linkage disequilibrium) indicates a polymorphism belonging to a group of polymorphisms which were in significant and complete linkage disequilibrium (D' = 1.000, r^2 = 1.000), and (*) indicates a polymorphism which was in significant linkage disequilibrium (D' = 1.000).

SNP in Table 2 indicates a polymorphism in the 3' untranslated region. The column of "TAA+" indicates the number of bases from a stop codon, TAA, in the mu-opioid receptor. "reported" indicates a genotype of a sequence registered in GenBank, and "major allele" and "minor allele" indicate a major genotype and minor genotype on the Japanese genome, respectively. The value in a parenthesis indicates the number of repeats of the base before the value. For example, "A(6)" means that A is repeated 6 times. "-" means deletion or insertion. "*" in the column of "LD" (Linkage disequilibrium) means that it is in significant and complete linkage disequilibrium (D' = 1.000, r^2 = 1.000).

Table 3 shows examples of nucleotide repeat polymorphisms of the mu-opioid receptor gene in normal subjects.

Table 3: Nucleotide repeat polymorphism of mu-opioid receptor gene in normal subject

	number of repeats	11	12	13	14	15						
IVS3+6113 (GT)n	control (2n=358)	35	1	284	33	5						
	(%)	(9.8)	(0.3)	(79.3)	(9.2)	(1.4)						
	number of repeats	2	-	8	9	10	11	12	13	14	15	16	17
IVS3+8761 (32bp)n	control (2n=358)	34	-	1	12	100	33	118	18	9	6	25	2
	(%)	(9.5)		(0.3)	(3.4)	(27.9)	(9.2)	(33.0)	(5.0)	(2.5)	(1.7)	(7.0)	(0.6)

The numerical value in a parenthesis indicates a percentage of the total number.

In Table 3, "IVS3+6113(GT)$_n$" means a polymorphism in which, assuming that the base which is in intron 3 and in contact with exon 3 is 1, G and T located 6113 and 6114 bp downstream thereof are repeated n times. In addition, "IVS3+8761(32bp)$_n$" means a polymorphism in which one unit composed of 32 bp located 8761 bp downstream in the same manner in intron 3 is repeated n times.

The polymorphisms shown in Tables 1, 2 and 3 are included in the mu-opioid receptor gene polymorphisms of the present invention. Further, oligonucleotides

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containing the gene polymorphisms of the present invention are shown in Table 4. For example, the oligonucleotide of the present invention is an oligonucleotide selected from nucleotide sequences represented by SEQ ID NOS: 1 to 98 containing any of the above-mentioned polymorphisms and nucleotide sequences complementary to any of these (Table 4).

Table 4

Position	Name of gene polymorphism	Sequence	SEQ ID NO
5' UTR	A-5580G	gttcaactgtcataccttagcaggaatcgaaacagtgacccatggcat a/g ctaagagtcactgtactcttcacagagctgcactcacagagaaaaacac	1
	C-5342T	actaaagtagaatgcttgtcccaaagaaaagcggcatgttgctgtttgag c/t tgtgaactaaattaaccactttttccgtggatcactattttattttaaag	2
	A-5308G	atgttgcctgtttgagctgtgaactaaattaaccactttttccgtggatc a/g ctattttattttattaaagaatgactggcggcggggccggtggctcacgcctg	3
	C-5236T	ctggcgcaggcgcggtggctcacgcctgtaatcccagcactttgggagg c/t tgaggcaggcagatcgacgagtcgaggagatcgagaccatcctggctaaca	4
	G-5026A	actcgggaggctggaggcttgcagtgagccgagatcgttttatgagctcctca g/a cctgggcgacagagtgagactctgtttaaaataaataaataaataaaat	5
	G-4936A	ataaataaataaaataaactgataagggaatgtttttatgagctctca g/a tttaattctgaagtgatgagactgtgtagaagtaacctaaataagaaa	6
	C-4504A	taattccttgctaattttctggccactacaacaggatataaaaagcc c/a aacaacaaaggataaattctttcatatgtgttgtaatcctaaacctct	7
	T-2694G	taaaatatgctaatcattttccaactgaattcaaatattatgcacatt t/g aatattcatatatgtttaatatagaaagaaacacagagtgaggagga	8
	A-2693C	aaaatatatgctaatcattttccaactgaattcaaatattatgcacatt a/c atattcatatatgtttaatatagaaagaaacacagagtgaggagga	9
	T-2683C	ctaatcattttccaactgaattcaaatattatgcacattacatatccata t/c atgttaatatagaaagaaacacagagtaggaggagtgcacatg	10
	T-2402C	aaaactatagtgttgtactgagctccctccaaagcaactataatta t/c aggagtgaaacatgattcacacggcataaagacaaagtttccgtaat	11
	C-1609T	tcccatgaactaagcaaaggaactgagtcagccagcagacagattcag c/t tcaataagagaattgttacattcatggaagaatatgttttaagg	12
	G-696T	tgtttctcatttcttttcagaaaataaagatcgctgttgttcccaaca g/t gtttgtaggaagaaaatggagaaacattattaccttttcttagatgtt	13
	G-490T	tagggtttcatcaggccaatgttattccctgccagatttaaggagaaa g/t gcgctggaaaatgagtgagtgatgttagcccccttctctattttcactgcta	14
Exon 1	A118G	cccccagccagcccccggtcctgggtcaacttgtcccacttgatggc a/g acctgtccgacccatgccgtccgaacgcaccgacctggcgcggagac	15
Intron 1	IVS1-A9080G	aatgaaaggccagaaaattagcccaaagagatgaaactcttcctcc a/g tcaccattgactctattgtgaacttatgaaaaggtagttggcaatatg	16
	IVS1-A9010G	gaacttatgaaaaggtagttggcaatatgaaggccatgatgtggaatt a/g aacacacacacacacacacacacacacatgctggattctcaaa	17
	IVS1-4908(AC)n	acttatgaaaaggtagttggcaatatgaaggccatgatgtggaattaa (ac)n atgctggattctaaaatgtcctcctcctcctcttgatcagtt	18
	IVS1-G4690A	acagaggtaatttatttgttctggctccattaaccacaaatagtcaaa g/a caatcacattttgaagtagtaatagttgggaaatgtgtgaagaatagg	19
	IVS1-T45910G	ggtcaaaagataaataagaattatttttataaccataagaaaggagaaca t/g ctataaacaaaagtcatatatgcaacctaaaagaataggtcagctgccag	20
	IVS1-T44560	ttctgtgagttccataaaaatcaactctaatgggtcaaacatcgatggttc t/g cagaagaacacaatttttcaaaaacgaatagcattgtaaattcatttg	21
Intron 2	IVS2-G631A	tacaacaaaatacaggcaagttagtgatgttaccagcctgaggaggga g/a ggttcacagcctgatatgttggtgatgtcataagcaaagcagtatttatg	22
	IVS2-G518A	tttatatcaatagacctatggaggatctagctcatgttgagagttc g/a ttttgttcctgaacgaaagccttaatgtgtcgaagtggactgcaaaat	23
	IVS2-G691C	ttccacaatttctttatagccttaagttagctctgtcaaggctaaaaat g/c aatgagcaaaatggcagtattaaccaccttatgacataattaaatgttgct	24
Intron 3	IVS3-G5607A	ctctaattactattaaagcactttctggacattttaatcaaaatagc g/a ggtcaagaagttaggagatgtctgtatttgttttaactgtgaactatat	25
	IVS3-G5953A	acatcactctcaaagttgtatctcagtttttttaccagacatctgtgga g/a agttaatttgggaaagtaatagttcaatggaaaaaaactcaa	26
	IVS3-6113(GT)n	atcaaaatggctattcttcagttctacagttttaaaagaaaatgttcc (gt)n ggtgtgatataggccatgtctctttttgcatgtatggaattagagtaat	27
	IVS3-A6151G	aaagaaaatggttccgtgtgtgtgtgtgtgtgtgtgtgtatag a/g catgtctctttttgcatgtatggaattagagtaaatgtaggtttaaaatt	28
	IVS3+A84490	tgatatataacaataagaattatttataataatatcataac a/g tgtattatcatattatgatcatcaataacatattatacatattatacatattac	29
	IVS3+C8497T	acatgtattatcatattgtatatataacatatatatcatatcatat c/t aggatataaatcatcacattttcaatcattgtttctgttaataagaca	30
	IVS3+8761(32bp)n	tatgacatatcaatatatatatattatcatattatgacatatcgtaatat (attatcatatatgacatatcataatat)n atcaaaagtca	31
	IVS3-G8004A	cagagctcatgcagcgcccagtcatccccattgccagtg	
		aatatatattatcatatgacatatcataatatatattatcatt g/a tgacatatatcataataataatatcaaaagtcacagagctcatgcaagccc	32
3' UTR	TAA+G886A	taaatgtactctttatttctcactggtttctccatactgcaggctcccc g/a catattatcttttttaactcagctcagaatccttatgcctttgaa	33
	TAA+T1360C	atctaggtagacacgccaagtcagtgatgcccatgcctagaagctgtccatt t/c tgaactttgtcagcatgattaaaagaatcaaatacctgtagttatct	34
	TAA+T1371C	cagccaagtcagatggccatggccatgctcctgagctctcattgaactttg t/c cagcattgattaaaagaatcaaatacctgtagttatctatgatgataca	35
	TAA+G1670A	ttatgtggactcaaccaacgtatccagtagatgggaaaaaaagaaagccca a/g aataggttttagtgttcctctgatgaagttcatgttcatgttgcttgtaa	36
	TAA+G1709A	aacaaaagccaaaatagttttttagtgttcctctgtgatgaagttcat g/a ttgctgtaataatccatttctcagcaatcattgtttctgcttaatagaca	37
	TAA+C2007T	atgcttttcatgagctaggatggtgttttcccaagagatgacatagtattg c/t tttgctcctcaagctgtttctcagcaatcattgtttctgcttaatacca	38
	TAA+A2109G	gctcctagtacggaattacatggtcattgtcaagttggagcaacttgtcttcaagt a/g ggacctgatctatctttttccacaaagtcatgtgtgaacaagtttct	39
	TAA+A2274G	attctaaagtaataataaataaggtcattgtcaagcttttcattcaaa a/g ccattttttaacgtaaattgctcggaaccaccttccaattccaaggcagg	40

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Table 4-2

Position	Name of gene polymorphism	Sequence	SEQ ID NO
	TAA+G2287A	taataataagtcatgtcaaagtttttcattcaaaaccattttttaac g/a taaattgctagaaccacttccaattccaaggcaaggagagagacattaca	41
	TAA+G2395C	ctcaactggtaggggctaaggtttctgataaaatcgaagataaagaaat g/c gaatattctgctttttcttccttctaatttcaccccttgcctaaggatga	42
	TAA+G2458C	tttttcttcctctaatttcacccctgcctaaggatgagattcttccca g/c gttggtatcccagaaatgcagactagctatgtgggcggaagctttgttt	43
	TAA+T2482C	ttgctaaggatgagatttcttccaggttgtatcccagaaatcagcac t/c gtagctatgggcggaagcttgtttcttacctgctcactgctgtgga	44
	TAA+G2497A	atttcttcccaggttgtatcccagaaatgcagactagctatggggcg g/a aagctttgtttcttcttacctgctcactgctgtggaaattctagcttattg	45
	TAA+G2656T	tccctcttttcctgccaatcattagaaggaaggaaggaaagagactc g/t ctggagcactggtgagtctctaggaccctgctatcctatcccaacagggc	46
	TAA+C2714A	actggtgagtctctaggaccctgctatcctatcccaacaggcgtgtcaga c/a ggagaactcctaatgtggccattgaaacactctcaaacattgaataga	47
	TAA+G2820T	gaagtttaaaataacctcttctaagcacaggctatgaggtaggtaaggaa g/t cattcattcccttcaataatgactgtgttgataaaactgataaccatt	48
	TAA+G2907T	aactgataaccattcacttgcaaatgttattatggaacaggtctcactta g/t ctcatttaattaccaaaagatgctaacaaattctgttcccacattg	49
	TAA+T3423C	gccaagcaacctaagaataggacatggtagcttaagttttcagctct t/c aactggacgacacacaagttgtttgtacaattcttgggtcaatc	50
	TAA+A4026G	caaacaatattacgtgtttctaagcgttctgttactcgaagggtctg a/g_ tccagacccaaagagagttcttgacctcatgcaagaaagaattcagg	51
	TAA+4565(A)n	ggtttgttttaagtaagccacttcctccctgcaagttccacacggacag (a)_ ggaggaaactttttcctggagcccactaatcaacagtgaacaaaaggc	52
	TAA+A4861C	taagaaagcaaggaataaagaatggctactccataggccagcgtagcccc a/c agggctgctggttggctattttttggttattcttgattataatgctaa	53
	TAA+A5359G	gtcgctcgttcaaacacctcgacactgaattacaataataaggacc a/g ttgacactgagatttaaggaggagaaaaagagattgacagtggactaag	54
	TAA+A6074C	gcaaggtaagaatcaagtagaaaatagtaaaggcgaggaaaaagatgaa a/c gctactcatattaaccattctaccattggattattgccaacacacct	55
	TAA+T6866G	gacagtgggaaaattcatcttcatattgtcacatgcactgtaatagaa t/g gtttagcaaaaaaaaccttccagagagaaggtggttccaatattacctac	56
	TAA+C6922G	gcaaaaaaacctccagagaaggtggttccaatattacctacaactt c/g ctttgcatttgatttttgaaggacctaaagttgaaaacaggctatca	57
	TAA+T705del (322bp)	taaatgtttattttaagtttgcatgcccactaaggctagaacatttttt del (322bp*) gataaattcacaggttacaaaataccaaacggaaatgagataagtggta	58
	TAA+C7427T	ggccggctagacatttttgataattcacagggttacaaaataccaaa c/t ggaatgagataagtggtaaaaccacagaagatataggagaagagaaaa	59
	TAA+7483del (A)	tgagtaagtggtataaccacagaagatataggggaggaagagaaaaaa del (a) gaggaaataaagaagagacaactctttcctaagagtctggtaaaattgaa	60
	TAA+T7530C	ggaataaagaagagacaactctttcctaagagtctgggtaaaattgaaca t/c agccatattcactgaacaacatgagtgagctcattaatttaagcacagc	61
	TAA+A7589G	ccatattcactgaacaacatgagtggctcattaagttaattaagcacagc a/g actgcttaattaacaagaccagagatgaaggaggagagagactacatttgt	62
	TAA+C8116T	gtgacatattagacttacttccccaataaaaaagtgcctgctggg c/t gcgtggctcacgccgtaattcccagcacttcgggagcccgagcggcg	63
	TAA+C8165T	gcgcggtggctcacgcctgtaattcccagcacttgggaggccgaggcggg c/g ggaacacaaggtcaggagatcaagaccatcctgccaatatggtaaaacc	64
	TAA+G8281A	atacaaattaggaagggggtggtggtgcacgcctgtaatcccagctagtc g/a ggaggctgaggcgaggagagattgcttgaactgggaggcggaagttgcagt	65
	TAA+G8386(A)n	caagatcgcagcattgcactccagcctggcaacagagatgagattgtctc (a)_ gtgccacatgccatgctatgtgcccaagttccttcacacaacagcc	66
	TAA+C9000T	ttagagccagtcagaattcaatctccaatatcctgactagacaagaaat c/t catagattgattcttgttctcctgcatctctgcaggtggcaaacctgatt	67
	TAA+A9564G	tcttcctaactaaatcttatcataagcaaatctatgcaccaaattattta a/g ggtgggttaattctgactacgggattcctttttcacttttataatgaac	68
	TAA+G9669A	ttagtacaattcctaataacagctgaaggaccatttatttgaagcaatg a/g tacaattcctaataacagctgaggaccatttattgaagcaatgttcac	69
	TAA+T9716A	ttgcccatgaatgtgacatgcatattaaatatggcacctctttaa t/g tcttttttctcataataagtttgaaactcacagtgagaaattgagaga	70
	TAA+T9839G	tggttgttctcgaactagctggttcccagacgacgtggagacggagca g/a ataagaactacattgagaagaaggctataagaactggacgtgaggagctg	71
	TAA+C9994A	catgggagctgagggtctgataaatgggaactgccagttaatagctatg c/a tattctgcataaataataaaaactagtattgttttcttctagctcgtttt	72
	TAA+C10083A	taatgttaaattggctctataaacataagtcaatttgctctattatgtc (a)m gagaataggagttttaacttatctgtgttttattaatattttgaagta	73
	TAA+10223(A)n	ataagtcaatttgctctattatgtcaaaagagaataggagttttaactt a/t tatctgtgtttattaatattttgaagtataggaaacctcatggtgtagca	74
	TAA+A10247T	gtatgtgcagggggctgcatgcaccggtggtctgggaggaacagacagg a/g caggagttcttcatttacaataagagaacagacaatgttcttctatacaa	75
	TAA+A10535G	agcagagcggcctggttttggttttactgaatatgaaacaacatacaa a/g ttttacttcctgttgttttactgaatatgaaacaatcatatatgatggag	76
	TAA+G10704A	tggttttacttcctgttgttttactgaatatgaaacaaacaaacaacca t/g gtagaggtcttctctcctctcaatgtcaacatcatatatgatggag	77
	TAA+T10752G	gctggtttggttgaagttttctctatcagtcaggcactttgcatttaag c/t gtactttaccacgcgacacctccccccc c/a agcacacacacacacacacacacacacacacacacacagtgaaatgg	78
	TAA+C11100T	...	79
	TAA+G11129A	tcaggcactttaagttaaggtgtactttaccaccacgcacacctccccccc c/a agcacacacacacacacacacacacacacacacacagtgaaatgg	80

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Table 4-3

Position	Name of gene polymorphism	Sequence	SEQ ID NO
	TAA+T11132(CA)n	ggcactttgcatttaagcgtactttaccaccgacacctccccccccag (ca)n acatagtgaaatggacccgtggaattatgatagttgtaatcaaaata	81
	TAA+A11336	gcactttgcatttaagcgtactttaccaccgacacctccccccccagc a/g cacacacacacacacacacacaacacatagtgaaatggaccc	82
	TAA+T11368del(TCTC)	tctggagtaaacttaaaatgaaattgaattgcttcaattatacta del(tctc) tatctaaatcttaattgaaattaaatattgtctctacccaaacca	83
	TAA+T11411C	tatcctatctctctaaatcttaattgaaattaaatattgtctc t/c acccaaaccatcgatttcatggaaatgttaaatttcttttttttttt	84
	TAA+T11431C	cttaattgaaattaaattatttgtctctacccaaaccatcgatttca t/c ggaaatgtttaaatttcttttttttttttgatggagtctcact	85
	TAA+T11449ins(TTTC)	tattttgtctctacccaaaccatcgattcatggaaatgttaaatttc ins(tttc) ttttttttttttttgatggagtctcactctgccaggctggag	86
	TAA+G11541A	aggctgcagtgcagtggcgcaatcttggctcactgcaacctctgcctcc g/a ggttcacaccattctcctgcttcagcctcagctgcctgggactacagg	87
	TAA+A11602C	attctcctgcttcagcctcctgagtagctggactacaggtgccgccac a/c acacctggctaattttttgtattttttagtagagatggggtttcaccacgt	88
	TAA+C11650T	acaacaacctggctaattttttgtattttttagtagagatggggttcacca c/t gttagccaggatggtttcgatctcctgacctcgtgatctgcctcggcctgg	89
	TAA+C11918T	atccccatcaatttaataggaattagttagaaatactagtatatatt c/t ccttatatactaattgtatatccatataaaagcattagtaccattatat	90
	TAA+A11956C	agtatatattcccttatatactaattgtatatccatataaaagcatt a/c gtaccattatatgaagtatatatgccattccataaaaatatatctacca	91
	TAA+A12143G	ggaattaaagaaaaatgcctgtttcactagtcatcctccctggca a/g tacatttcctgaactttacatactaaatagccagttatgaaaatgtaa	92
	TAA+A12630G	acattttaaacagacctcctgcccacaacatatttttcctctccaggaata a/g gaatgcaactgaattgccttcctccttcacccattgtcctctttactgcttat	93
	TAA+T12681C	gaatggcaactgaattgttccttctttatctatagcttaagtcaaacc t/c aacataagcaateaaccttccacccattgtcctctttagctgcttat	94
	TAA+T12831C	ttggggtgaaataaaagatagaccccctgctgctctgcacgtagattcag t/c ttgtatgccagggtgacatttaattacagtgtcagacacctaaaca	95
	TAA+C12834C	ggggtgaataaaagatagaccccctgctgctgcacgtagattctttgg g/c tatgccagggtgacatttaattacagtgtccatcatcatgtctgtttactattgaaaa	96
	TAA+T13236(T)n	caacattgttttcctttgatgctgggagtttctataagtttttg (t)n ctcttcattagtgtgttagttccatcatcatgtctgtttactattgaaaa	97
	TAA+T13971G	ttgaaatataggcagctaaatcactgatagtctactttttaaaaat t/g tgttcttgtgtttgagcaggaaaaatatttgcaagaacaaaagagttt	98

*1 322 bp deletion
ttttttttttttttttttttttggacacagctcgctctgtgcccaggctggagtacagtggcgggatctcggctc
actgcaagctccgcctcccgggttcacgccattctcctgcctcagcctcccaagtagctgggactacaggcgcccgccac
tacgccggctaatttttgtatttttagtagagacgggggtttcacgtttagccaggatgtctcgatctcctgacct
cgtgatccgcccgcctcggcctcccaaagtgctggattacaggcgtgagccaccgcgcccggccggcccagacattttt

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In Table 4 (SEQ ID NOS: 1 to 98), 101 bases are shown, and a polymorphic site is shown at the 51st base. One represented by "a/g" means a polymorphism associated with "a" and "g", and "c/t" means a polymorphism associated with "c" and "t". Further, "$(ac)_n$" means a polymorphism in which "ac" is repeated n times, "del(a)" means a deletion polymorphism of "a", and "ins(tttc)" means an insertion polymorphism of "tttc". Other bases are indicated in the same manner. Among these, in the present invention, an oligonucleotide having at least 10 bases, preferably 10 to 100 bases, more preferably 10 to 45 bases, further more preferably 14 to 25 bases containing a polymorphic site (the 51st base) in any of the nucleotide sequences (SEQ ID NOS: 1 to 98) shown in Table 4 and an oligonucleotide complementary to this are provided. Here, the "51st base" means a base at a polymorphic site, and the base is not necessarily one base. Accordingly, the base is not present in a deletion polymorphism, therefore, the base is decided not to be counted in the number of bases, and even if a plurality of bases are repeated, they are decided to be counted as the "51st base".

Incidentally, in Table 4, a sequence of 322 bp contained in a gene polymorphism represented by "TAA+7075del(322bp)" (SEQ ID NO: 58) is shown below and

outside the table.

3. Haplotype analysis

In the present invention, by using SNP among the above-mentioned gene polymorphisms, a haplotype can be constructed. The SNP to become a target of a haplotype analysis may be any as long as its polymorphism frequency is 0.5% or higher, preferably, those with a polymorphism frequency of 1%, more preferably those with a polymorphism frequency of 5% or higher can be selected. Further, SNP to become a target of a haplotype analysis may be a full or partial sequence thereof.

The haplotype analysis can be carried out using various computer programs, and for example, Arlequin program (available from http://anthro.unige.ch/arlequin) (Schneider S, Roessli D, Excoffier L. Arlequin 2000: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept of Anthropology Univ of Geneva.) can be used.

As an example of the haplotype analysis, as for the 10 sites of SNPs with a polymorphism frequency of 5% or higher among polymorphisms found as in the above-mentioned 2, a haplotype is estimated using Arlequin program. At this time, as for the groups of SNPs showing complete linkage disequilibrium present in a

region from intron 3 to the 3' untranslated region, one of them, for example, "TAA+A2109G" can be used as a representative. The estimated haplotypes are shown in Table 5.

Table 5: Haplotype of mu-opioid receptor gene polymorphism in normal subjects

No.	frequency	A118G	IVS2 +G691C	IVS3 +G5807A	IVS3 +G5953A	IVS3 +A6151G	IVS3 +A8449G	IVS3 +C8497T	TAA +A2109G	TAA +A2274G	TAA +G2287A
1	4.9%	A	C	A	G	G	A	C	A	A	A
2	3.0%	A	C	G	G	G	A	C	A	A	A
3	24.6%	A	C	G	G	G	A	T	A	A	A
4	6.5%	A	G	G	A	G	A	C	A	G	A
5	5.4%	A	G	G	G	A	G	C	G	A	G
6	35.9%	G	C	A	G	G	A	C	A	A	A
7	3.6%	G	C	G	G	G	A	T	A	A	A
8	16.1%	27 haplotypes with lower frequencies less than three percents									
sum	100.0%										

As for the haplotypes of the mu-opioid receptor gene polymorphism in normal subjects, 34 haplotypes are estimated, and among these, there are 7 haplotypes observed at a high frequency of 3%, and 27 haplotypes which is estimated to occur at a frequency of 3% or lower are collectively shown in No. 8 of Table 5.

Subsequently, based on the above-mentioned haplotype frequency, a linkage disequilibrium analysis is carried out. The D' value and r^2 value, which indicate measures of linkage disequilibrium, can be calculated based on the following definition.

Definition:

Assuming that there are SNP A and SNP B, and the respective alleles are represented by A and a, and B and b. The four haplotypes formed by SNP A and SNP B are represented by AB, Ab, aB and ab, and the respective haplotype frequencies are represented by P_{AB}, P_{Ab}, P_{aB} and P_{ab}.

$D = P_{AB} \times P_{ab} - P_{Ab} \times P_{aB}$

(In the case of $D > 0$)

$D' = (P_{AB} \times P_{ab} - P_{Ab} \times P_{aB})/\text{Minimum}\ (((P_{AB} + P_{aB}) \times (P_{aB} + P_{ab})),\ ((P_{AB} + P_{Ab}) \times (P_{Ab} + P_{ab})))$

(In the case of $D < 0$)

$D' = (P_{AB} \times P_{ab} - P_{Ab} \times P_{aB})/\text{Minimum}\ (((P_{AB} + P_{aB}) \times (P_{AB} + P_{Ab})),\ ((P_{aB} + P_{ab}) \times (P_{Ab} + P_{ab})))$

$r^2 = (P_{AB} \times P_{ab} - P_{Ab} \times P_{aB})^2/\{(P_{AB} + P_{Ab})(P_{AB} + P_{aB})(P_{aB} + P_{ab})(P_{Ab} + P_{ab})\}$

[However, Minimum $(((P_{AB} + P_{aB}) \times (P_{aB} + P_{ab})),\ ((P_{AB} + P_{Ab}) \times (P_{Ab} + P_{ab})))$ means that a smaller value among $(P_{AB} + P_{aB}) \times (P_{aB} + P_{ab})$] and $((P_{AB} + P_{Ab}) \times (P_{Ab} + P_{ab}))$ is adopted.]

Table 6 shows one example showing linkage disequilibrium among mu-opioid receptor gene polymorphisms in normal subjects

Table 6: Linkage disequilibrium among mu-opioid receptor gene polymorphisms in normal subjects

D'

Locus	A118G	IVS2 +G691C	IVS3 +G5807A	IVS3 +G5953A	IVS3 +A6151G	IVS3 +A8449G	IVS3 +C8497T	TAA +A2109G	TAA +A2274G	TAA +G2287A
A118G		0.795	0.704	0.626	0.866	0.884	0.633	0.875	0.458	0.890
IVS2 +G691C	0.125		1.000	0.714	0.746	0.795	0.775	0.772	0.672	0.795
IVS3 +G5807A	0.453	0.134		0.921	0.849	0.883	0.895	0.868	0.535	0.883
IVS3 +G5953A	0.052	0.341	0.102		0.023	0.009	1.000	0.034	0.742	0.009
IVS3 +A6151G	0.054	0.201	0.047	0.000		1.000	1.000	1.000	1.000	1.000
IVS3 +A8449G	0.069	0.284	0.063	0.000	0.001		1.000	1.000	1.000	0.967
IVS3 +C8497T	0.159	0.062	0.291	0.069	0.037	0.046		1.000	0.763	1.000
TAA +A2109G	0.061	0.241	0.055	0.001	0.892	0.901	0.042		1.000	1.000
TAA +A2274G	0.024	0.266	0.030	0.484	0.011	0.014	0.035	0.014		1.000
TAA +G2287A	0.070	0.284	0.063	0.000	0.800	0.934	0.046	0.989	0.014	

r^2

In the results of the linkage disequilibrium analysis, a combination with a D' value (upper right of the table) being significant and 0.7 or higher is underlined, and a combination with an r^2 value (lower left of the table) being significant and 0.5 or higher is represented by a bold and italic form.

In Table 6, when the loci are named beginning at the top such that A118G is named "SNP1", IVS+G691C is named "SNP2", and so on, and TAA+G2287A is named "SNP10", the linkage disequilibrium between SNP1 and SNP2 is calculated and the resulting value is written in the cell at the intersection of the row of SNP1 and the column of SNP2 (upper right of the table). The value of D',

which is an index of the linkage disequilibrium, of 0.7 or higher is underlined. Further, an r^2 value, which is a more stringent index of the linkage disequilibrium, is calculated in the same manner, and the resulting value is written in the cell at the intersection of the row of SNP1 and the column of SNP2 (lower right of the table).

When attention is given to D' in Table 6, except for IVS3+G5953A and TAA+A2274G, significant linkage disequilibrium has been observed in all the combinations of SNPs. Further, when attention is given to r^2 values, significant linkage disequilibrium can be observed in three SNPs in intron 3 and the 3' untranslated region, and one of them is TAA+A2109G.

Further, in the present invention, the linkage disequilibrium between a repeat polymorphism and each SNP can also be analyzed. For example, the repeat polymorphisms shown in Table 3 exhibit diversity, however, significant linkage disequilibrium between them and all the 10 SNPs shown in Table 6 could be observed ($P < 0.05$).

Further, in the present invention, from the results of the linkage disequilibrium analysis (Table 6), four haplotype blocks can be estimated. As for the haplotype block, from the results of the haplotype analysis, for example by using HaploBlockFinder Version 0.6, a linkage

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block can be estimated (http://cgi.uc.edu/cgi-bin/ kzhang/haploBlockFinder.cgi).

From this estimation, the SNPs shown in Table 6 can be classified into 4 haplotype blocks, i.e., a block containing A118G, a block containing IVS2+G691C and IVS3+G5807A, a block containing IVS3+G5953A and a block composed of SNP downstream of IVS3+A6151G.

When a specific SNP in the estimated haplotype blocks is examined, the information of SNPs indirectly linked to each other in the same block can be obtained. That is, when a gene polymorphism of the mu-opioid receptor is examined, it is not necessary to analyze all the SNPs, and it is only necessary to perform typing for several specific SNPs.

4. Correlation between mu-opioid receptor gene polymorphism and drug sensitivity

It is considered that when a polymorphism occurs in the mu-opioid receptor gene, the expression level or function of mu-opioid receptor changes. Therefore, there is a correlation between a mu-opioid receptor gene polymorphism and various phenotypes associated with the mu-opioid receptor in some cases. Here, as the phenotype, a phenotype associated with occurrence of a disease and a phenotype associated with drug sensitivity can be

exemplified.   As   the   phenotype   associated   with
occurrence of a disease, pain sensitivity, vulnerability
to drug addiction and the like can be exemplified.   As
the   phenotype   associated   with   drug   sensitivity,   an
efficacy of a drug, a side effect of a drug, duration
of efficacy of a drug and the like can be exemplified.

A correlation between a mu-opioid receptor gene
polymorphism and a phenotype can be examined as follows.
As for the mu-opioid receptor gene polymorphism, as a
result of the linkage disequilibrium analysis and the
haplotype analysis in normal subjects, a representative
polymorphism   in   the   estimated   linkage   blocks,   for
example SNP is selected.   Then, a polymorphism frequency
is   analyzed   for   this   polymorphism   (such   as   SNP)   in
individual   test   subjects   (patients),   and   comparison
thereof with a polymorphism frequency of normal subjects
is carried out.   In the comparison, it is effective that
a statistical technique such as a chi-square test is used.

In the case where the phenotype or the symptom is
a stimulant-induced psychotic-like symptom, it can be
classified,   for   example,   according   to   a   period   of   time
from the start of the use of a stimulant to the occurrence
of delusion or hallucination, a period of duration of
delusion or hallucination after termination of the use
thereof, the presence or absence of the relapse, and the

presence or absence of multiple drug abuse. Then, for each class, the polymorphism frequencies or genotypes of normal subjects and test subjects are compared. As a result, a polymorphism with a significant difference in the polymorphism frequency compared with the control group can be used for evaluating the susceptibility to a disease or the difference in drug sensitivity. However, it is suggested that the tendency of gene polymorphism is affected by the race, birthplace or the like, it is preferred that in a group showing a similar gene polymorphism to that of a population used for finding an associated polymorphism (such as SNP), the above-mentioned evaluation using the polymorphism is carried out.

5. Use of analysis results

The results analyzed as described above can be used as an index in a method of predicting sensitivity to various drugs associated with the mu-opioid receptor, a method of selecting a method of treating or preventing a disease associated with the mu-opioid receptor, a method of determining an appropriate administration amount of a therapeutic drug, a method of predicting side effects or the like. Incidentally, by determining the genotype of an individual with the use of the

oligonucleotide or the microarray of the present invention, it becomes possible to perform the prospective evaluation of drug sensitivity.

As the drug associated with the mu-opioid receptor (function modulator), a stimulant such as methamphetamine, a dopamine receptor agonist, a dopamine receptor antagonist, a μ-, κ-, or δ-opioid receptor agonist, a μ-, κ-, or δ-opioid receptor antagonist, alcohol and the like can be exemplified, and examples thereof may include methamphetamine methylenedioxymethamphetamine, amphetamine, dextroamphetamine, dopamine, morphine, DAMGO, codeine, methadone, carfentanil, fentanyl, heroin, cocaine, naloxone, naltrexone, nalorphine, levallorphan, pentazocine, buprenorphine, oxycodone, hydrocodone, levorphanol, etorphine, dihydroetorphine, hydromorphone, oxymorphone, ethanol, methanol, diethyl ether, tramadol and the like.

Further, by using the gene polymorphism or the method of the present invention, it is possible to evaluate drug sensitivity or the like in different races. The subjects are not particularly limited and examples thereof include Japanese, Europeans, Americans and the like, however, in the present invention, they are preferably Japanese or those having a similar gene

polymorphism tendency to that of Japanese.

Morphine is used as an analgesic drug, however, it also has an effect on suppressing respiration, therefore it is necessary to determine its administration amount carefully. If a correlation between the novel gene polymorphism which was elucidated by the present invention and the sensitivity to morphine has been analyzed and each individual has been determined in advance for what polymorphism confers what sensitivity to morphine in it, by examining a mu-opioid receptor gene polymorphism of a patient to whom morphine is to be administered, the sensitivity to morphine of the patient is elucidated and an administration amount can be determined.

A genome sample of a test subject can be extracted from the blood, saliva, skin or the like, however, the origin is not limited to these as long as a genome sample can be collected therefrom. The extraction and purification methods of genomic DNA is publicly well known. For example, genomic DNA is purified from a specimen such as the blood, saliva, skin or the like collected from a human using the phenol method or the like. At this time, a commercially available genomic DNA extraction kit such as GFX Genomic Blood DNA Purification Kit or a device may be used. In the case where SNP to

be examined is in an open reading frame, mRNA or total RNA may be extracted instead of genomic DNA. Hereinafter, an example of the gene polymorphism detection method used for the above-mentioned test sample will be shown.

(1) Detection using the PCR method

In order to amplify a test sample by PCR, it is preferred that a high fidelity DNA polymerase, for example, KOD Dash polymerase (TOYOBO) is used. A primer to be used is designed such that a gene polymorphism is contained at a given position of the primer so as to amplify a target SNP in the test sample and synthesis is carried out.

After completion of the amplification reaction, detection of the amplified products is carried out, and the presence or absence of a polymorphism is determined.

(2) Detection by the sequencing method

In the present invention, the polymorphism of the present invention can also be detected by a sequencing method based on the dideoxy method. As a sequencer to be used for the sequencing, a commercially available ABI series (Amersham Biosciences) is used.

(3) Detection by a DNA microarray

A DNA microarray is a microarray in which a nucleotide probe has been immobilized on a support, and includes a DNA chip, a Gene chip, a microchip, a bead

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array and the like.

First, a polynucleotide of a test sample is isolated and amplified by PCR, and then labeled with a fluorescent reporter group. Then, a labeled DNA/mRNA, total RNA are incubated along with an array. Then, this array is inserted in a scanner, and a hybridization pattern is detected. The data of the hybridization is collected as emitted light from the fluorescent reporter group bound to the probe array (i.e., incorporated in a target sequence). A probe which is completely identical with the target sequence generates a stronger signal than those having a region which is not identical with the target sequence. Because the sequence and the position of each probe on the array are known, the sequence of the target polynucleotide reacted with the probe array can be determined based on the complementarity.

(4) Detection by the TaqMan PCR method

The TaqMan PCR method is a method utilizing an allele specific oligonucleotide labeled with fluorescence and a PCR reaction with Taq DNA polymerase. The allele specific oligonucleotide (referred to as TaqMan probe) to be used in the TaqMan PCR method can be designed based on the above-mentioned gene polymorphism information.

(5) Detection of gene polymorphism by the invader method

The invader method is a method of detecting a gene polymorphism by subjecting an allele specific oligonucleotide and a template to hybridization. A kit for carrying out the invader method is commercially available, and it is possible to easily detect a gene polymorphism by this method.

6. Kit

In the present invention, an oligonucleotide containing a mu-opioid receptor gene polymorphism (such as SNP) can be included in a kit for detecting a gene polymorphism.

The kit for detecting a gene polymorphism of the present invention includes one or more components necessary for carrying out the present invention. For example, the kit of the present invention includes a component for storing or supplying an enzyme and/or a reaction component necessary for detecting a gene polymorphism. Such a component is not limited, however, examples thereof include the oligonucleotide of the present invention, an enzyme buffer solution, dNTP, a reagent for control (such as a tissue sample or a target oligonucleitide for a positive or negative control), a reagent for labeling and/or detection, a solid phase support, a written instruction manual and the like.

Further, the kit of the present invention may be a partial kit including a part of the necessary components. In this case, a user can prepare the other components.

The kit of the present invention can be provided as a microarray in which the above-mentioned oligonucleotide has been immobilized on a support. The microarray is one in which the oligonucleotide of the present invention has been immobilized on a support, and includes a DNA chip, a Gene chip, a microchip, a bead array and the like.

The kit of the present invention includes an oligonucleotide containing a gene polymorphism found in the present invention. Therefore, the blood is collected before a drug is applied to a patient or the like (for example, before surgery, at the time of occurrence of cancer pain or the like), and a mu-opioid receptor gene is isolated. Then, this gene is reacted with an oligonucleotide in the kit, whereby a genotype is determined. From the determined genotype, a dosage regimen such as the type or dose of the drug is formed. As a result, an effect of the drug suitable for an individual can be obtained, which is useful in the personalized medicine. For example, in the case of using morphine, it becomes possible to obtain an analgesic effect suitable for an individual, and also to suppress

the side effects to the minimum.

Hereinafter, the present invention will be described in more detail with reference to Examples, however, the invention is not limited to Examples.

[Example 1] SNP analysis and haplotype construction
(1) Subjects

The present Example was carried out by using as subjects, 128 methamphetamine-addicted patients (99 males, 29 females, the average age of 35.9 ± 1.0) who show F15.2 and F15.5 based on the diagnostic criteria ICD-10 DCR of WHO and were hospitalized in or had been released from a psychiatric hospital or department of psychiatry in Japan belonging to a study group of analyzing genome associated with drug addiction (Japanese Genetics Initiative for Drug Abuse (JGIDA)), and 179 normal subjects (139 males, 40 females, the average age of 34.6 ± 1.5) who were mainly engaged in a medical service and did not have a history of drug addiction in the past or did not have any family member who had a history of drug addiction, and did not suffer from a mental disease.

Further, as a result of analysis of polymorphism frequency in normal subjects, SNP was observed with a frequency which was significantly different from the

polymorphism frequency reported so far in exon 1 and intron 2, therefore, as for these two regions, 34 and 53 normal subject samples were added, respectively and an analysis was carried out.

The diagnosis was carried out based on an interview by two psychiatrists and all the available information including the records of the hospital. Further, patients with a history of schizophrenia, organic psychosis syndrome, or another mental disease were excluded from the addicted patients.

The test subjects were all Japanese, and their birthplaces and places of residence were Kitakyusyu, Setouchi, Tokai and Kanto regions.

The present Example was carried out after the approval by ethical review in each institute belonging to JGIDA was obtained and a written consensus on the use of the genome sample in this study was obtained by all the test subjects.

(2) Analysis for cases classified according to stimulant-induced psychotic-like symptoms

In an analysis performed after classifying the methamphetamine-addicted patients based on the clinical information, the patients were classified into groups in terms of the following 4 items.

(A) Latency of psychosis (the period of time from the

start of the use to the occurrence of delusion or hallucination)

• Occurred within 3 years (n = 54, the average number of years = 0.83 year)

• Occurred after a lapse of not less than 3 years (n = 53, the average number of years = 9.98 years)

(B) Prognosis of psychosis (the period of duration of delusion or hallucination after termination of the use)

• transient (n = 72, disappeared within one month)

• middle (n = 17, disappeared within a period of one month to 6 months)

• prolong (n = 26, continued for not less than 6 months)

(C) Spontaneous relapse (the presence (n = 86) and the absence (n = 42) of relapse)

(D) Poly drug abuse (multiple drug abuse)

• none (n = 36, single abuse of methamphetamine)

• easily accessible drug only (n = 49, with a history of abusing alcohol or thinner)

• heavy (n = 43, with a history of abusing morphine, heroin, cannabis or the like)

Further, the methamphetamine-addicted patients for whom the clinical information in terms of the respective items could not be obtained were excluded from this analysis.

(3) Genotyping

A genome sample was purified from a blood specimen collected from each test subject using a standard phenol extraction method. More specifically, to the whole blood, 3-fold volume of a hemolysis reagent (0.1 mM EDTA, 155 mM NH_4Cl_2) was added, and the mixture was left at room temperature for 10 minutes and then centrifuged. Then, the precipitate was resuspended. The resulting suspension was subjected to a proteinase K treatment and an RNase treatment and then centrifugation. Then, from the resulting supernatant, phenol/chloroform extraction was carried out, followed by ethanol precipitation, whereby genomic DNA was prepared. By using the genomic DNA as a template, the PCR method was carried out using KOD Dash (TOYOBO, Tokyo), and the resulting product was purified by ethanol precipitation, then a template DNA for sequencing was prepared.

As for a 5' side untranslated region (5' UTR) and 5' flanking region of a mu-opioid receptor gene, a full region of interest, that is, a region up to 5765 bp upstream from the start codon was amplified by carrying out PCR amplification of two regions (-5765 to -2272 and -2612 to 421). As for exon 1, exons 2 to 3 (containing intron 2), exon 4 (containing a region 2952 bp downstream from the stop codon) and a part of introns 1 and 3,

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amplification of DNA fragment was carried out by the PCR method for each region (see Fig. 1 for the amplified regions). As for a 3' side untranslated region (3' UTR and 3' flanking region), a full region of interest, that is, a region up to 13830 bp downstream from the stop codon was amplified by carrying out PCR amplification of four regions (TAA+2004 to 6288, 4980 to 10376, 8292 to 12120, and 11260 to 14677). The base sequence was decoded by the sequencing method with the use of Big Dye Terminator V3.1 Cycle Sequencing Ready Reaction (Applied Biosystems, Tokyo) by using these PCR products as templates and an analysis of gene polymorphisms was carried out.

First, by using 44 cases of the genome samples of normal subjects, for the full region of the amplified PCR fragments, a polymorphism observed at a high frequency on a mu-opioid receptor gene was found with the use of the 152 primers in total. Then, with regard to the entire exon region and a region containing a polymorphism observed at a high frequency (allelic frequency > 5%), by using the genomic DNA of the remaining normal subject samples and methamphetamine-addicted patient samples, an analysis of mu-opioid receptor gene polymorphism was carried out. The primers were designed with reference to gene sequences published in National Center of Biotechnology Information (GenBank Accesion

No. NT-023451).

(4) Statistic analysis

A significant difference test for polymorphism frequency between normal subject samples and methamphetamine-addicted patient samples was carried out using a chi-square test (significance level $\alpha = 0.05$). Further, in order to confirm the bias of samples for all the polymorphisms, examination of Hardy-Weinberg (HW) equilibrium was carried out using a chi-square test. As for a linkage disequilibrium analysis and a haplotype analysis, the genotype information of 179 cases of normal subjects and 128 cases of methamphetamine-addicted patients was analyzed using Arlequin program (available from http://anthro.unige.ch/arlequin) (Schneider S, Roessli D, Excoffier L. Arlequin 2000: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept of Anthropology Univ of Geneva). Then, a D' value and r^2 value, which indicate the measures of linkage disequilibrium, were calculated based on the definition described above.

The significant difference of polymorphism frequency between normal subject samples and methamphetamine-addicted patient samples in a repeat polymorphism was obtained using CLUMP program produced based on the Monte Carlo method (Sham PC, Curtis D. Monte

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Carlo tests for associations between disease and alleles at highly poly morphic loci. Ann Hum Genet, 59, 97-105, (1995)).

From the results of the haplotype analysis, a linkage block was estimated using HaploBlockFinder Version 0.6.

[Example 2] Analysis of mu-opioid receptor gene polymorphism in Japanese genome

In order to identify a mu-opioid receptor gene polymorphism, by using the genomic DNA of Japanese normal subjects, the sequence was decoded after PCR amplification for an entire exon region, a part of an intron region, and the 3' and 5' untranslated regions. With regard to the entire exon region and a part of an intron region, a polymorphism analysis was carried out for a region shown with 7 lines in the lower portion of the genomic schematic view of Fig. 1. Further, with regard to A118G, IVS2+G691C, a group of SNPs being in complete linkage disequilibrium found in a wide range from intron 3 to around 4 kbp downstream of the 3' UTR, and a dinucleotide repeat polymorphism, IVS3+6113 (GT)$_{11-15}$ (Example 3) and 32-nucleotide repeat polymorphism, IVS3+8761 (32bp)$_{2-17}$ (Example 3), a linkage disequilibrium analysis was carried out based on the

polymorphism frequency of normal subject samples, and a D' value among polymorphisms exhibiting significant linkage disequilibrium is shown in Fig. 1 (P < 0.05).

In the decoded regions, 38 novel gene polymorphisms in total, i.e., 36 polymorphisms in Table 1 and 2 polymorphisms in Table 3, and three SNPs (A118G, IVS2+G31A and IVS2+G691C), which had been already reported in studies using other races were found on the Japanese genomes (above Table 1).

Among 39 SNPs shown in Table 1 found in Japanese, majority of them, i.e., 37 SNPs were transversion mutations, and 2 sites (IVS2+G691C and TAA+G2395C) were transition mutations. Further, it was confirmed that because all these polymorphisms exhibited Hardy-Weinberg (HW) equilibrium, there was no bias among collected samples. On the Japanese genomes in the present Example, 14 SNPs were confirmed in the 5' untranslated region, however, 24 SNPs which had been reported in the same region before were not found.

Further, with regard to two SNPs, i.e., A118G and IVS2+G691C, their polymorphism frequencies ("Allelic frequency" in Table 1) in the Japanese genomes were 45.3% and 81.9%, respectively, while their polymorphism frequencies which had been reported mainly in the European and American genomes ("Reported Allelic

frequency" in Table 1) were 7.5 to 25.8% and 42.5 to 53.3%, respectively. The two SNPs were found at a significantly high frequency in the Japanese genomes (Hoehe MR, Kopke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, et al. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Hum Mol Genet, 9, 2895-908, (2000)).

Further, SNP present in other translated regions such as C17T, which had been reported to be found at a high polymorphism frequency in a study using other races before, was not observed, either. (Hoehe MR, Kopke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, et al. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Hum Mol Genet, 9, 2895-908, (2000)、Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci U S A, 95, 9608-13, (1998)、Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, et al. Mu opioid receptor gene variants: lack of association with alcohol dependence. Mol Psychiatry, 2, 490-4, (1997)、Sander T,

Gscheidel N, Wendel B, Samochowiec J, Smolka M, Rommelspacher H, et al. Human mu-opioid receptor variation and alcohol dependence. Alcohol Clin Exp Res, 22, 2108-10, (1998)、Gelernter J, Kranzler H, Cubells J. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. Mol Psychiatry, 4, 476-83, (1999)、Franke P, Wang T, Nothen MM, Knapp M, Neidt H, Albrecht S, et al. Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. Am J Med Genet, 105, 114-9, (2001)、Shi J, Hui L, Xu Y, Wang F, Huang W, Hu G. Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. Hum Mutat, 19, 459-60, (2002)、Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, et al. A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. Mol Psychiatry, 7, 224-8, (2002))

Further, what is interesting is that polymorphisms were found at substantially the same time in some of the SNPs found in intron 3 and the 3' untranslated region, and they showed a D' value of 1.000 in the linkage disequilibrium analysis. Then, in order to examine the size of this linkage disequilibrium block, when the

number of cases was reduced (5 cases each for 3 genotypes),
and the analyzed range was expanded to about 14 kbp
downstream from the stop codon (TAA), additional 57 gene
polymorphisms were newly found. The polymorphisms found
in the 3' untranslated region of a mu-opioid receptor
gene and the linkage disequilibrium thereof are shown
in the above Table 2.

Among the newly found 57 gene polymorphisms, 24 gene
polymorphisms were observed at the same time as the
above-mentioned group of polymorphisms being in linkage
disequilibrium, and were in complete linkage
disequilibrium showing both D' value and r^2 value of 1.000
in the linkage disequilibrium analysis (a symbol "*" in
Table 2).

[Example   3]   Haplotype   analysis   and   linkage
disequilibrium analysis for mu-opioid receptor gene
polymorphism

Among the polymorphisms found in Example 2, with
regard to 10 sites of SNPs with a polymorphism frequency
of 5% or higher, a haplotype was estimated using Arlequin
program (above Table 5). When a haplotype was estimated,
with regard to the group of SNPs showing complete linkage
disequilibrium present in a range from intron 3 to the
3' untranslated region among the SNPs with a polymorphism

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frequency of 5% or higher, TAA+A2109G, which was one of the SNPs, was used as a representative. By using Arlequin program, 34 haplotypes were estimated, and among these, there were 7 haplotypes observed at a high frequency of 3% or higher (No. 1 to No. 7 in Table 5). Those with a frequency of 3% or lower (the remaining 27 haplotypes, which corresponds to 16.1% of the total haplotypes) are collectively shown in No. 8.

The G allele of the above-mentioned TAA+A2109G was observed only in the No. 5 haplotype, and some of the other SNPs also had a similar tendency.

Then, based on the obtained haplotype frequency, a linkage disequilibrium analysis was carried out (above Table 6).

In Table 6, among the results of the linkage disequilibrium analysis, a combination of polymorphisms showing a D' value (upper right of the table) being significant and 0.7 or higher is underlined, and a combination with an r^2 value (lower left of the table) being significant and 0.5 or higher is represented by a bold and italic form. When the D' value was calculated as defined above, significant linkage disequilibrium was observed in all the combinations of SNPs except for some of the combinations related to IVS3+G5953A or TAA+A2274G (Table 6).

Further, as for the r^2 value, which is a more stringent index, significant linkage disequilibrium was observed in three SNPs in an intron and the 3' untranslated region, and one of the three SNPs was TAA+A2109G, which is one of the above-mentioned groups of polymorphisms showing complete linkage disequilibrium (Table 6).

Further, from the results of the haplotype analysis, four linkage blocks were estimated.

In addition, on the mu-opioid receptor gene, several nucleotide repeat polymorphisms other than SNPs were newly found. In intron 3, a dinucleotide repeat polymorphism, IVS3+6113 $(GT)_{11-15}$ and 32-nucleotide repeat polymorphism, IVS3+8761 $(32bp)_{2-17}$ were observed (above Table 3).

Although these repeat polymorphisms exhibit great diversity, significant linkage disequilibrium between them and all the 10 SNPs shown in Table 6 was observed $(P < 0.05)$.

[Example 4] Correlation between mu-opioid receptor gene polymorphism and methamphetamine addiction or stimulant- induced psychotic-like symptoms

In order to examine a correlation between mu-opioid receptor gene polymorphisms and methamphetamine

addiction or stimulant-induced psychotic-like symptoms, four sites of SNPs (A118G, IVS2+G691C, IVS3+A6151G and IVS3+G8497T), which are representatives of the SNPs in the linkage blocks estimated at a polymorphism frequency of 5% or higher from the results of the linkage disequilibrium analysis and the haplotype analysis in normal subjects (Example 3) were selected.

First, a polymorphism frequency in all the methamphetamine-addicted patients was analyzed, and compared with that of normal subjects (Table 7).

Table 7: Comparison of polymorphism frequencies of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients

Locus	Normal subjects				Methamphetamine-addicted patients					
	Genotypic data			Allelic data		Genotypic data				Allelic data	
	number	allelic frecuency		number		number		allelic frecuency	P value	number	P value
A118G	A 67 (0.31) A/G 99 (0.46) G 47 (0.22) total 213	45.3%		A 233 (0.55) G 193 (0.45) total 426		A 49 (0.38) A/G 54 (0.43) G 25 (0.19) total 128		40.6%	P=0.43	A 152 (0.59) G 104 (0.41) total 256	P=0.23
IVS2 +G691C	G 6 (0.03) G/C 72 (0.31) C 154 (0.66) total 232	81.9%		G 84 (0.18) C 380 (0.82) 464		G 12 (0.09) G/C 43 (0.33) C 73 (0.57) total 128		73.8%	P=0.012	G 67 (0.26) C 189 (0.74) total 256	P=0.011
IVS3 +A6151G	A 2 (0.01) G/A 23 (0.13) G 154 (0.86) total 179	92.5%		A 27 (0.08) G 331 (0.92) total 358		A 1 (0.01) G/A 26 (0.20) G 101 (0.79) total 128		89.1%	P=0.21	A 28 (0.11) G 228 (0.89) total 256	P=0.15
IVS3 +C8497T	C 85 (0.47) C/T 76 (0.42) T 18 (0.10) total 179	31.3%		C 246 (0.69) T 112 (0.31) total 358		C 73 (0.57) C/T 42 (0.33) T 13 (0.10) total 128		26.6%	P=0.209	C 188 (0.73) T 68 (0.27) total 256	P=0.21

In Table 7, only those with a polymorphism frequency

significantly different from that of normal subjects by the comparison are shown. The polymorphism frequency of IVS2+G691C in the methamphetamine-addicted patients is 73.8%, which is lower than 81.9% of the normal subjects. Therefore, it was elucidated that the genotype is significantly different (P = 0.023, chi-square test), and even in the comparison of allelic frequencies, C allele occurred at a high frequency, which is significantly different from normal subjects (P = 0.023, chi-square test).

Further, also in IVS3+A8497G, it was confirmed that there is a tendency in that the ratio of G allele is higher in the methamphetamine-addicted patients than that of the normal subjects.

Subsequently, as for the above-mentioned four SNPs, from the information of gene polymorphisms of the methamphetamine-addicted patients, a haplotype was estimated using Arlequin program (Table 8), and a linkage disequilibrium analysis was carried out based on the resulting haplotype frequency, which was compared with that of normal subjects (Table 9).

Table 8: Comparison of haplotype frequencies of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients

	Control frequency	MAP dependent frequency	Haplotype			
			A118G	IVS2 +G691C	IVS3 +A6151G	IVS3 +C8497T
1	1.4%		A	C	A	C
2	0.2%		A	C	A	T
3	10.9%	11.0%	A	C	G	C
4	24.3%	23.2%	A	C	G	T
5	5.7%	10.9%	A	G	A	C
6	9.2%	12.7%	A	G	G	C
7	1.7%	1.5%	A	G	G	T
8	40.0%	37.8%	G	C	G	C
9	4.9%	1.8%	G	C	G	T
10	0.3%		G	G	A	T
11	1.5%	0.9%	G	G	G	C

Table 9: Comparison of linkage disequilibrium of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients

D'

r^2

Locus	A118G	IVS2 +G691C	IVS3 +A6151G	IVS3 +C8497T
A118G		0.795 0.897	0.866 1.000	0.633 0.794
IVS2 +G691C	0.125 0.195		0.746 1.000	0.775 0.739
IVS3 +A6151G	0.054 0.084	0.201 0.346		1.000 1.000
IVS3 +C8497T	0.159 0.156	0.062 0.071	0.037 0.044	

In Table 9, among the results of the linkage disequilibrium analysis, a combination of polymorphisms

showing a D' value (upper right of the table) being significant and 0.7 or higher is underlined. In the respective combinations of the table, the upper numerical values indicate the results of the linkage disequilibrium analysis for the genomes of the normal subjects and the lower numerical values indicate the results of the linkage disequilibrium analysis for the genomes of the methamphetamine-addicted patients.

Table 9 shows the results of the linkage disequilibrium analysis of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients, and a combination of polymorphisms showing a D' value (upper right of the table) being significant and 0.7 or higher is represented by a dark-gray color. In the respective combinations of Table 9, the upper numerical values indicate the results for the genomes of the normal subjects and the lower numerical values indicate the results for the genomes of the methamphetamine-addicted patients. Further, in all the combinations in the linkage disequilibrium analysis in the four SNPs, the D' values exhibited a significant value, however, any of the r^2 values did not exhibit a significant value (Fig. 9).

Although a significant difference was not observed (P = 0.40) with regard to the haplotype frequencies

between the normal subjects and the methamphetamine-addicted patients, in the No. 5 and the No. 6 haplotypes in Table 8, the polymorphism frequency of the methamphetamine-addicted patients showed a tendency of being higher compared with that of the normal subjects. The difference between the No. 5 and the No. 6 haplotypes was only a difference in the allele type of IVS3+A6151G, which is one of the groups of SNPs showing complete linkage disequilibrium present in a region from intron 3 to the 3' untranslated region.

Accordingly, it can be said that there is a possibility that an additional haplotype analysis may show a useful difference for addiction to other drugs, pain therapy and the like.

[Example 5] Analysis for cases classified according to stimulant-induced psychotic-like symptoms

In the present Example, comparison of polymorphism frequencies of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients classified according to stimulant-induced psychotic-like symptoms was carried out.

Methamphetamine-addicted patients were classified as described in Example 1 according to the cases of the stimulant-induced psychotic-like symptoms based on the

clinical information, and an analysis was carried out. The analysis was carried out for all the four sites of SNPs (A118G, IVS2+G691C, IVS3+A6151G and IVS3+G8497T), which are representatives of the linkage blocks estimated from the linkage disequilibrium analysis in Example 3. Among them, only polymorphisms in which a group with a polymorphism frequency significantly different from that of normal subjects is present are shown in Table 10.

Table 10: Comparison of polymorphism frequencies of mu-opioid receptor gene polymorphisms between normal subjects and methamphetamine-addicted patients classified according to stimulant-induced psychotic-like symptoms

| Locus | Normal subjects | | Methamphetamine-addicted patients | | | | | |
	Number of cases	Polymorphism frequency	group 1 Number of cases	Polymorphism frequency	group 2 Number of cases	Polymorphism frequency	group 3 Number of cases	Polymorphism frequency
(1) *1			Less than 3 years		3 years or more			
A118G	A 67 (0.31) A/G 99 (0.46) 45.3% G 47 (0.22) total 213		25 (0.46) 24 (0.44) 31.5% P= 5 (0.09) 0.0404 54		20 (0.38) 25 (0.47) 38.7% P= 8 (0.15) 0.4691 53			
IVS2 +G691C	G 6 (0.03) G/C 72 (0.31) 81.9% C 154 (0.66) total 232		6 (0.11) 20 (0.37) 70.4% P= 28 (0.52) 0.0085 54		5 (0.09) 19 (0.36) 72.6% P= 29 (0.55) 0.0394 53			
IVS3 +A6151G	A 2 (0.01) G/A 23 (0.13) 92.5% G 154 (0.86) total 179		0 (0.00) 15 (0.28) 86.1% P= 39 (0.72) 0.0269 54		1 0.019 7 0.132 91.5% P= 45 0.849 0.9060 53			
(2) *2			transient		middle		prolonged	
IVS2 +G691C	G 6 (0.03) G/C 72 (0.31) 81.9% C 154 (0.66) total 232		8 (0.11) 27 (0.38) 70.1% P= 37 (0.51) 0.0034 72		0 (0.00) 5 (0.29) 85.3% P= 12 (0.71) 0.7807 17		3 (0.12) 8 (0.31) 73.1% P= 15 (0.58) 0.0596 26	
(3) Existence of relapse			not exist		exist			
IVS2 +G691C	G 6 (0.03) G/C 72 (0.31) 81.9% C 154 (0.66) total 232		6 (0.07) 30 (0.35) 75.6% P= 50 (0.58) 0.1250 86		5 (0.12) 14 (0.33) 71.4% P= 23 (0.55) 0.0143 45			
(4) Multiple drug abuse			none		easily accesible drug		heavy	
IVS2 +G691C	G 6 (0.03) G/C 72 (0.31) 81.9% C 154 (0.66) total 232		5 (0.14) 11 (0.31) 70.8% P= 20 (0.56) 0.0059 36		3 (0.06) 15 (0.31) 78.6% P= 31 (0.63) 0.4401 49		1 (0.02) 18 (0.42) 76.7% P= 24 (0.56) 0.3801 43	
IVS3 +C8497T	C 85 (0.47) C/T 76 (0.42) 31.3% T 18 (0.10) total 179		24 (0.67) 6 (0.17) 25.0% P= 6 (0.17) 0.0137 36		24 (0.49) 22 (0.45) 28.6% P= 3 (0.06) 0.6986 49		22 (0.51) 17 (0.40) 29.1% P= 4 (0.09) 0.9104 43	

*1: Period of time of the occurrence of delusion or hallucination
*2: Period of duration of delusion or hallucination

The analysis was carried out for all the four sites of SNPs, which are representatives of the linkage blocks

estimated from the linkage disequilibrium analysis of normal subjects, and among them, only polymorphisms in which a group with a polymorphism frequency significantly different from that of normal subjects is present are shown in Table 10.

(1) When classification was carried out according to the period of time from the start of the use of a stimulant to the occurrence of delusion or hallucination, in a group in which a symptom occurred within 3 years, the polymorphism frequencies of three sites of SNPs (A118G, IVS2+G691C and IVS3+A6151G) showed a lower value compared with the normal subjects, and the genotypes were significantly different (P = 0.040, P = 0.0085, P = 0.027, respectively). With regard to IVS2+G691C, also in a group in which delusion or hallucination occurred after a lapse of not less than 3 years from the start of the use of methamphetamine, the genotype was significantly different in the same manner (P = 0.039) compared with the normal subjects, however, a group, in which the period of time up to the occurrence of symptom is shorter, showed a tendency that the genotype is more significantly different.

(2) When classification was carried out according to the period of time from the stop of the use of a

stimulant to the disappearance of delusion or hallucination, in a group in which a symptom relatively promptly disappeared within 1 month, the polymorphism frequency of IVS2+G691C showed a lower value, and the genotype was significantly different compared with the normal subjects (P = 0.0034).

(3) Also in the case where classification was carried out in terms of the presence or absence of the relapse, in a group in which relapse was observed, the polymorphism frequency of IVS2+G691C showed a lower value, and the genotype was significantly different compared with the normal subjects (P = 0.014).

(4) When classification was carried out using a multiple drug abuse as an index, and an analysis was carried out, in a group with a history of only methamphetamine abuse, the polymorphism frequencies of two sites of SNPs (IVS2+G691C and IVS3+C8497T) showed a lower value, and the genotypes were significantly different compared with the normal subjects (P = 0.0059, P = 0.014).

In the Japanese samples, a polymorphism present in a translated region was present in exon 1, and was only A118G which changes asparagine to aspartic acid. It is considered that this asparagine is one site among five glycosylation sites present at the N-terminus of the

mu-opioid receptor, which is a seven-transmembrane receptor.

In the present Example, in the comparison of polymorphism frequency of A118G between the normal subjects and the methamphetamine-addicted patients, a significant difference was not observed in the same manner as the past reports (Table 7), however, when the methamphetamine-addicted patients were classified according to stimulant-induced psychotic-like symptoms, the polymorphism frequency was significantly different compared with the normal subjects in some of the groups (Table 10).

When the methamphetamine-addicted patients were classified based on the clinical information and an analysis was carried out, in the period of time of the occurrence of delusion or hallucination, the period of duration and the presence or absence of the relapse, a group in which the polymorphism frequency was significantly lower compared with the normal subjects was observed in some of the SNPs. This indicates that a group with a major allele is liable to develop stimulant-induced psychosis caused by methamphetamine (Table 10).

The present invention shows that a gene polymorphism of the mu-opioid receptor, in particular,

IVS2+G691C can be considered as one of the useful gene markers associated with vulnerability to methamphetamine addiction and stimulant-induced psychosis. Further, it shows that a difference in some regulatory mechanism for the expression of the mu-opioid receptor due to a gene polymorphism other than a translated region greatly contributes to various individual differences in an action mediated by the mu-opioid receptor.

Industrial Applicability

According to the present invention, a novel mu-opioid receptor gene polymorphism is provided. By using the polymorphism of the present invention, individual differences in sensitivity to a drug can be evaluated. By this method of evaluating sensitivity, an appropriate prescribed amount of a narcotic drug such as morphine can be found.

Sequence listing free text

SEQ ID NO: 18: n represents m-time repeat of ac (existing position: 51)

SEQ ID NO: 27: n represents 11- to 15-time repeat of gt (existing position: 51).

SEQ ID NO: 31: n represents 2- to 17-time repeat

of   attatcatattatgacatatatcataatatat   (existing position: 51).

SEQ ID NO: 52: n represents m-time repeat of a (existing position: 51).

SEQ ID NO: 58: n represents a 322-bp sequence or deletion (existing position: 51).

SEQ ID NO: 60: n represents a or deletion (existing position: 51).

SEQ ID NO: 66: n represents m-time repeat of a (existing position: 51).

SEQ ID NO: 74: n represents m-time repeat of a (existing position: 51).

SEQ ID NO: 81: n represents m-time repeat of ca (existing position: 51).

SEQ ID NO: 83: n represents tctc or deletion (existing position: 51).

SEQ ID NO: 86: n represents insertion of tttc or does not represent anything (existing position: 51).

SEQ ID NO: 97: n represents m-time repeat of t (existing position: 51).